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Abstract

Objectives:

To investigate whether (1) there were differences between HLA-DRB1*15-positive and -negative patients at baseline, and (2) HLA-DRB1*15-positive patients showed a greater development of brain and spinal cord damage, as assessed by MRI, and greater progression of disability, during a 5-year follow-up, compared with HLA-DRB1*15-negative patients.

Methods:

HLA-DRB1*15 typing was performed in 41 patients with primary progressive multiple sclerosis (PPMS) who were recruited within 5 years of symptom onset. All patients and 18 healthy controls were studied clinically and with MRI at baseline, and every 6 months for 3 years, and then at 5 years. Magnetization transfer ratio parameters and volumes for brain gray matter and normal-appearing white matter, brain T2 lesion load, and spinal cord cross-sectional area were obtained. Patient disability was assessed at each visit using the Expanded Disability Status Scale and Multiple Sclerosis Functional Composite subscores.

Results:

There were no significant differences between HLA-DRB1*15-positive and -negative patients at baseline. HLA-DRB1*15-positive patients showed a greater decline in brain magnetization transfer ratio for gray matter and normal-appearing white matter (both $p = 0.005$) than HLA-DRB1*15-negative patients over 5 years, while the same parameters did not change over time in healthy controls. HLA-DRB1*15-positive patients also showed a trend toward a faster increase in brain T2 lesion load than HLA-DRB1*15-negative patients (0.29 [95% confidence interval 0.20–0.38] vs 0.21 [0.13–0.30] mL/mo, $p = 0.085$) and higher T2 lesion volumes at all time points (average difference [95% confidence interval]: 10.58 mL [7.09–14.07], $p < 0.001$) during the follow-up, after adjusting for disease duration.

Conclusions:

These findings suggest that HLA-DRB1*15 influences the progression of brain pathology in PPMS.

The allele HLA-DRB1*15 has been associated with susceptibility to multiple sclerosis (MS).1 It also seems to influence the phenotypic expression of the disease, regarding age at onset,2 cognitive disability,3 and extent of brain damage at a single time point, as detected by conventional and advanced MRI.4 A recent postmortem study suggests HLA-DRB1*15 is associated with a greater extent of spinal cord pathology in MS, mainly demyelination and inflammation.5

The rate of progression of disability and accumulation of brain and spinal cord damage differs among patients with primary progressive multiple sclerosis (PPMS), especially in the first few years after onset.6,–8 It is unknown whether these rates are associated with specific genetic factors. The identification of these factors may be useful for stratification of patients at higher risk of progression.

The question that we address in this report is whether HLA-DRB1*15 influences the rate of progression and the development of brain and spinal cord damage, as detected by conventional and advanced MRI, in patients with early PPMS during a 5-year follow-up.

Methods

Study design.

Forty-seven patients with definite or probable PPMS9 within 5 years of symptom onset were studied with clinical and brain MRI assessments at baseline and every 6 months for 3 years, and again at 5 years. Spinal cord MRI was performed at baseline and year 2. Eighteen healthy controls were scanned at all time points. At each visit, patients were scored on the Expanded Disability Status Scale (EDSS).10 In 15 patients, the EDSS score at 5-year follow-up was not obtained in person but assessed by phone, because patients were too disabled to attend their visits. When possible, the Multiple Sclerosis Functional Composite subtests (i.e., the timed 25-foot walk test, the 9-Hole Peg Test, and the Paced Auditory Serial Addition Test) were also scored3 (see table 1 for patient characteristics at baseline).

HLA-DRB1*15 typing on total genomic DNA, extracted from whole blood, was performed in 41 of 47 patients as described previously.11

Image acquisition and processing.

All scans were performed on a 1.5-tesla GE Signa scanner (General Electric Co., Milwaukee, WI). During the study, the scanner was upgraded. The effect of the upgrade was considered in the statistical analysis.7

The following sequences were acquired at several time points, as previously reported6,7,12: (1) brain magnetization transfer dual-echo spin-echo sequence, including proton density and T2-weighted images13; (2) brain volumetric images (3-dimensional inversion prepared fast spoiled gradient recall); and (3) spinal cord volumetric imaging.

T2 lesion volume, magnetization transfer ratio (MTR) histogram parameters for gray matter (GM) and normal-appearing white matter (NAWM) (i.e., mean, peak location, and peak height [PH]), the percentage GM and NAWM fractions, and spinal cord cross-sectional area at C2-3 were obtained as previously described.6,14 See appendix e-1 on the Neurology® Web site at Neurology.org for further information on image acquisition and processing.

Statistical analysis.

Analysis was performed with Stata 12 statistical software (StataCorp, College Station, TX). Clinical and MRI differences between HLA-DRB1*15-positive and -negative patients at baseline were assessed using multiple linear regression, with MRI or clinical response variable as predictor on a patient group indicator; age, sex, or disease duration was entered as covariate to assess any potential confounding. Rates of change in brain and spinal cord MRI and clinical measures were compared between patient groups using linear mixed models. The linear mixed models specified a random intercept and random slope (on time), together with an unstructured covariance. The MRI or clinical variable was the response variable, and as predictors we included time from symptom onset, a subject group indicator, and a group × time interaction term. In addition, interaction terms between time and age, sex, or baseline EDSS score were added as covariates to control for potentially confounding baseline differences between the 2 patient groups. Where regression residuals showed deviation from normality, confidence intervals (CIs) and p values were obtained using bias-corrected nonparametric bootstrap with 1,000 replicates. An additional indicator for observations occurring after a scanner upgrade was entered to adjust for potential upgrade-induced change.

Standard protocol approvals, registrations, and patient consents.

The study was approved by the Joint Medical Ethics Committee of the National Hospital for Neurology and Neurosurgery and the Institute of Neurology, London, UK. We received written informed consent from all patients participating in the study.

Twenty-one patients with PPMS were HLA-DRB1*15 positive and 20 were negative. No statistically significant differences in clinical or MRI variables were found between these 2 patient groups at baseline (table 1).

Results

Both groups of patients progressed over time, as demonstrated by significant rates of changes in the EDSS score, 25-foot walk test, and 9-Hole Peg Test (table 2). There was a significant change in all of the brain MRI variables and spinal cord cross-sectional area over 5 and 2 years, respectively, in both the allele-positive and -negative patients, with the exception of the NAWM PH MTR, which did not significantly change in either group. Changes in NAWM peak location and mean MTR and GM PH MTR in the allele-negative patients did not reach statistical significance (table 2). None of these parameters changed significantly over time in healthy controls[7] (table e-1).

When comparing the rate of changes between HLA-DRB1*15-positive and -negative patients, the allele-positive patients showed a greater decline in brain PH MTR for GM and NAWM than HLA-DRB1*15-negative patients (GM: by $-1 \times 10-5$ percentage volumes/mo, 95% CI $-2 \times 10-5$ to $-0.51 \times 10-5$, vs $0.041 \times 10-5$, 95% CI $-1 \times 10-5$ to $1 \times 10-5$, p = 0.004; NAWM: by $-2 \times 10-5$ percentage volumes/mo, 95% CI $-4 \times 10-5$ to $0.12 \times 10-5$ vs

1 × 10−5, 95% CI −1 × 10−5 to 3 × 10−5, p = 0.004) when correcting for age and sex (table 2). We also repeated the analyses adjusting for T2 lesion volume at baseline, to rule out that differences in monthly rates of change over time between groups were due to the difference in baseline T2 lesion volume between the 2 groups, which was, however, nonsignificantly greater in allele-positive patients than in allele-negative patients (see table 1). After adjusting for T2 lesion volume at baseline, the differences between genetic groups in monthly rates in MTR measures were still significant (for GM PH MTR: p = 0.011; for NAWM PH MTR: p = 0.012). Similarly, the differences in monthly rates of changes in MTR measures remained significant when adjusting for baseline EDSS score (for GM PH MTR: p = 0.004; for NAWM PH MTR: p = 0.011). In addition, HLA-DRB1*15-positive patients showed a borderline significantly faster increase in brain T2 lesion volume than HLA-DRB1*15-negative patients over time (by 0.29 mL per month [95% CI 0.20–0.38] vs 0.21 [95% CI 0.13–0.30], p = 0.085) (figure, table 2). Also, this longitudinal model, which accounted for the disease duration of our patients, showed that allele-positive patients showed consistently higher T2 lesion volumes at all time points: average difference (along the whole follow-up) = 10.58 mL (95% CI 7.09–14.07), p < 0.001.

There were no significant between-group differences in the rates of changes in NAWM and GM volumes, spinal cord cross-sectional area, and clinical scores (table 2).

Discussion

In this study, we found that carriage of HLA-DRB1*15 was associated with increases in the development of brain GM and white matter pathology, as reflected by reduced MTR, a trend toward increased T2 lesion load over 5 years, and greater T2 lesion volumes at each time point over the follow-up, in patients with early PPMS.

At baseline, there were no statistically significant differences in MRI measures between HLA-DRB1*15-positive and -negative patients. However, allele-positive patients showed a more rapid MTR decrease over time than patients not carrying the allele (and healthy controls), indicating that allele-positive patients may have greater accumulation of brain microstructural damage over time. MTR reflects the amount of macromolecular structure within the CNS, through the quantification of the transfer of magnetization between macromolecule-bound and free water.15 MTR is sensitive to myelin content and axonal density,15 and a decrease in MTR can be expected where there is demyelination and axonal loss.15 Axonal loss, with a consequent decrease in both myelin content and axonal density, is a widespread pathologic process in the NAWM,16 and demyelinating lesions may be extensive in the cortical GM although not visible on MRI.17

Close scrutiny of the behavior of different MTR-derived histogram parameters demonstrated a greater decline in the PH MTR for both the NAWM and GM in allele-positive patients than in the negative ones. PH MTR indicates the volume of tissue with the most frequently observed MTR value (within that tissue). Thus, a PH MTR decrease probably indicates loss of tissue homogeneity secondary to microstructural changes, because a wider range of MTR values within that tissue is observed. The finding of reduced tissue homogeneity in the absence of significant changes in MTR peak location (possibly reflecting less pronounced structural damage) in the allele-positive patients compared with the allele-negative patients may reflect changes in the cellular composition of the tissue, such as an increased number

of activated microglial cells, which are known to be diffusely present in the NAWM and GM.18

In our study, allele-positive patients showed a trend toward a faster increase in T2 lesion volume over time than allele-negative patients, and greater T2 lesion volumes at all time points over the follow-up after adjusting for disease duration, despite not finding crude (unadjusted) differences in T2 lesion volume between genetic groups at baseline. This could be attributable to the increased precision of our longitudinal models, which considered the disease duration of every patient. It could also be attributable to the fact that, at baseline, patients at higher risk of developing more lesions over time (i.e., allele-positive patients) might not have had the time to show these differences as compared with allele-negative patients, especially considering that our follow-up was longer than the reported mean disease duration at baseline (5 vs 3.5 years). In addition, because of our sample size, it is likely that our study was underpowered to detect these (possibly very small) differences between groups.

We found that both genetic groups showed a significant development of brain atrophy over time, in contrast to healthy controls, who did not. However, there were no differences in the rates of GM or NAWM volume loss over time between genetic groups. Although somewhat surprising, these findings may have 2 interpretations. First, they suggest that MTR parameters are more sensitive to tissue damage progression over time than volume loss, at least in early PPMS.7 Second, the stronger association between MTR measures or T2 lesion volume and HLA genes may reflect a greater role of these genes in affecting inflammatory pathways with consequent demyelination and decrease in MTR, rather than affecting neurodegenerative processes (and hence no greater extent of atrophy). In line with this hypothesis, we did not find a relationship between cord atrophy over time and HLA-DRB1*15. Similarly, a recent study found an effect of HLA-DRB1*15 allele on inflammation and demyelination at the microscopic level, but not atrophy.5 In fact, the proteins encoded by HLA genes, which are considered as MS susceptibility genes, are involved in inflammatory pathways such as antigen presentation. In particular, the HLA-DRB1*15 allele is believed to enhance the presentation of myelin basic protein peptides to T cells, thus promoting the presence of autoreactive T cells against the myelin basic protein.4 In our study, there was no availability of spinal cord sequences to calculate the spinal cord lesion volume or number, so we limited our investigation of the spinal cord MRI to spinal cord cross-sectional area (or atrophy). Thus, we could not confirm the previously reported association between HLA-DRB1*15 allele and number of spinal cord lesions.19

Regarding the lack of significant brain atrophy over time among controls, it is possible that this not only reflected the absence of disease, but also the fact that the controls were younger, on average, than the patients.20

Despite a significantly greater decline in the brain GM and NAWM MTR in the allele-positive patients than in the allele-negative patients, there was no difference in disability progression between patient groups over time. This could be explained by the observation that in this cohort of early PPMS, MRI parameters predicted future disability7 rather than concurrent clinical changes, suggesting that a follow-up period longer than 5 years may be necessary to

detect the clinical impact of the observed MTR changes. There may also be other factors—genetic, epigenetic, or environmental—that influence neurodegeneration, and hence the rate of tissue loss and clinical progression, independently of HLA-DRB1*15 status. Finally, the clinical measures may lack power because of the relatively low sensitivity of currently used disability scales to clinical worsening. Moreover, longitudinal cognitive data, which reflect microscopic CNS tissue changes better than motor disability data,4,12 were not recorded, probably contributing to the absence of association between HLA-DRB1*15 status and disability. A possible limitation of this study, in addition to the rather small sample size, which implies a reduction in our power to detect significant differences between genetic groups, is that we could not explore a possible dose-response relationship between genotype and MRI features that would have supported causality because all allele-positive patients were heterozygous regarding HLA-DRB1*15.

Although in this study we report a number of statistical tests, we did not think it would be appropriate to adjust for multiple comparisons because we examined a number of separate null hypotheses rather than one single null hypothesis, meaning that the risk of false-positive results is not increased despite the amount of tests performed.21,22 Lastly, even though the linear mixed models we have used tend to be robust to the effect of missing data under plausible assumptions, it should be noted that more severely affected patients were more likely to be missing at MRI, and any remaining bias in our results attributable to this would tend to render our results conservative, because these missing patients were also more likely to be allele positive.

This study shows the influence of the HLA-DRB1*15 gene on the accumulation of microstructural damage in the brain, as detected in vivo by MTR and T2 lesions, at the earliest stages of PPMS, but not on brain atrophy and spinal cord atrophy. This finding might also have important practical implications, considering that patients with PPMS who have a greater risk of accumulation of brain pathology may need to be stratified for early and targeted therapeutic interventions.

Acknowledgements

The authors thank the subjects for kindly agreeing to take part in this study.

Glossary

CI	confidence interval
EDSS	Expanded Disability Status Scale
GM	gray matter
MS	multiple sclerosis
MTR	magnetization transfer ratio
NAWM	normal-appearing white matter
PH	peak height

PPMS primary progressive multiple sclerosis

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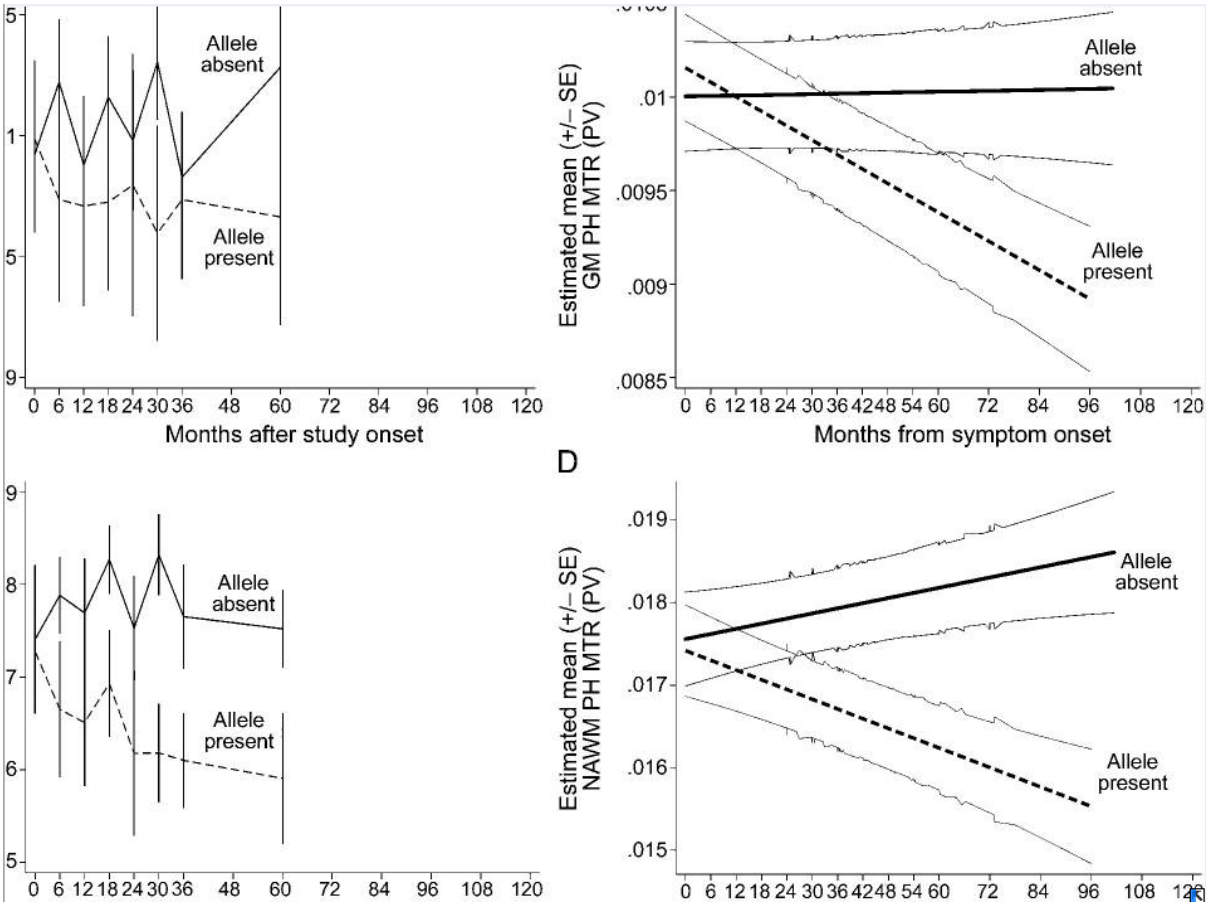
FIGURES

Figure. Raw and estimated (adjusted) rates of change in MRI measures in HLA-DRB1*15-positive and -negative patients

Raw (A, C) and estimated (B, D) values of GM PH MTR (in PV) (A, B), and NAWM PH MTR (in PV) (C, D) over time, for allele-positive and allele-negative patients. Allele-positive patients show a significantly greater decrease in PH MTR than allele-negative patients (p = 0.004 for both, GM and NAWM PH MTR). GM = gray matter; MTR = magnetization transfer ratio; NAWM = normal-appearing white matter; PH = peak height; PV = percentage volumes.



Tables

				Patients assessed genetically (n = 41)	
	Patients (n = 47)	Controls (n = 18)	p Value (patients vs controls)	Allele-positive (n = 21)	Allele-negative (n = 20)
Clinical and demographic characteristics					
Age, y	45.13 (10.66)	34.56 (5.79)	0.0002	42.90 (9.33)	47.65 (9.59)
Sex, no. of females (%)	19 (40.4)	10 (55.6)	0.2720	7 (33.3)	9 (45.0)
Disease duration, y	3.38 (0.8736)	—	—	3.38 (0.74)	3.45 (0.89)
EDSS, median (range)	4.5 (1.5–7.0)	—	—	6.0 (1.5–7.0)	4.5 (3.5–6.5)
TWT z score	−0.099 (1.224)	—	—	−0.102 (1.365)	0.065 (0.658)
9-HPT z score	-2.253×10^{-7} (1)	—	—	−0.014 (1.045)	0.238 (0.796)
PASAT z score	2.513×10^{-6} (1)	—	—	−0.014 (1.045)	−0.149 (1.011)
MRI measures					
T2LV, mL	30.257 (25.301)	—	—	34.621 (28.073)	22.756 (15.393)
GMPF, %	47.666 (2.262)	49.694 (1.356)	0.0010	47.350 (2.902)	48.113 (1.396)
NAWMPF, %	24.774 (2.317)	27.061 (0.924)	0.0002	24.955 (2.687)	25.141 (1.814)
GM mean MTR, PU	31.784 (1.139)	33.095 (0.435)	<0.0001	31.907 (1.297)	31.859 (0.960)
GM PL MTR, PU	33.339 (0.627)	33.818 (0.489)	0.0061	33.405 (0.578)	33.400 (0.651)
GM PH MTR, PV	0.010 (0.001)	0.012 (0.001)	<0.0001	0.010 (0.001)	0.010 (0.001)
NAWM mean MTR, PU	37.221 (0.901)	38.081 (0.457)	0.0004	37.337 (0.927)	37.377 (0.665)
NAWM PL MTR, PU	37.672 (0.813)	38.388 (0.448)	0.0011	37.814 (0.785)	37.753 (0.695)
NAWM PH MTR, PV	0.017 (0.003)	0.020 (0.001)	0.0002	0.017 (0.002)	0.017 (0.004)
Spinal cord area, mm^2	79.000 (8.053)	79.473 (9.225)	0.8670	79.909 (9.058)	78.273 (8.052)

Table 1. Clinical, demographic, and MRI characteristics of subjects at the study entry.

Abbreviations: EDSS 5 Expanded Disability Status Scale; GM 5 gray matter; GMPF 5 gray matter parenchymal fraction; MTR 5 magnetization transfer

ratio; NAWM 5 normal-appearing white matter; NAWMPF 5 NAWM parenchymal fraction; 9-HPT 5 9-Hole Peg Test; PASAT 5 Paced Auditory Serial

Addition Test; PH 5 peak height; PL 5 peak location; PU 5 percentage units; PV 5 percentage volumes; T2LV 5 T2 lesion volume; TWT 5 25-foot walk test.

Results are expressed as mean (SD), unless otherwise stated. No significant differences were observed between allele-positive and allele-negative patients.

	Patients assessed genetically (n = 41)	
MRI variables	Allele-positive (n = 21), rate of change (95% CI); *p* value	Allele-negative (n = 20), rate of change (95% CI); *p* value
T2LV, mL	0.290 (0.199 to 0.382); $p < 0.001$	0.215 (0.133 to 0.296)[b]; $p < 0.001$
GMPF, %	−0.023 (−0.031 to −0.014); $p < 0.001$	−0.021 (−0.034 to −0.007); $p = 0.002$
NAWMPF, %	−0.016 (−0.024 to −0.008); $p < 0.001$	−0.012 (−0.021 to −0.004); $p = 0.004$
GM mean MTR, PU	−0.015 (−0.021 to −0.009); $p < 0.001$	−0.009 (−0.015 to −0.003)[b]; $p = 0.003$
GM PL MTR, PU	−0.011 (−0.018 to −0.004); $p = 0.003$	−0.006 (−0.011 to −0.001); $p = 0.018$
GM PH MTR, PV	-1×10^{-5} (-2×10^{-5} to -0.514×10^{-5}); $p = 0.001$	0.0415×10^{-5} (-1×10^{-5} to 1×10^{-5})[c,d,e]; $p = 0.07$
NAWM mean MTR, PU	−0.004 (−0.007 to −0.002); $p = 0.002$	−0.001 (−0.005 to 0.002); $p = 0.442$
NAWM PL MTR, PU	−0.005 (−0.009 to −0.001); $p = 0.019$	−0.002 (−0.006 to 0.002); $p = 0.241$
NAWM PH MTR, PU	-2×10^{-5} (-4×10^{-5} to 0.123×10^{-5}); $p = 0.065$	1×10^{-5} (-1×10^{-5} to 3×10^{-5})[c,d,e]; $p = 0.397$
Spinal cord area, mm^2	−0.287 (−0.471 to −0.102); $p = 0.002$	−0.148 (−0.285 to −0.011); $p = 0.034$
EDSS	0.020 (0.011 to 0.029); $p < 0.001$	0.019 (0.006 to 0.031); $p = 0.009$
PASAT, s	−0.004 (−0.083 to 0.076); $p = 0.924$	−0.013 (−0.091 to 0.064); $p = 0.739$
1/TWT, 1/s	−0.001 (−0.0014 to −0.0005); $p < 0.001$	−0.001 (−0.0013 to −0.0004); $p < 0.001$
1/9-HPT, 1/s	−0.0001 (−0.0002 to −0.0001); $p < 0.001$	−0.0001 (−0.0001 to −0.00003); $p = 0.002$

Table 2. Monthly rates of change of clinical and MRI measures over the 5-year follow-up perioda and differences between HLA-DRB1*15-positive and -negative patients

Abbreviations: CI 5 confidence interval; EDSS 5 Expanded Disability Status Scale; GM 5 gray matter; GMPF 5 gray matter parenchymal fraction; MTR 5

magnetization transfer ratio; NAWM 5 normal-appearing white matter; NAWMPF 5 NAWM parenchymal fraction; 9-HPT 5 9-Hole Peg Test; PASAT 5

Paced Auditory Serial Addition Test; PH 5 peak height; PL 5 peak location; PU 5 percentage units; PV 5 percentage volumes; T2LV 5 T2 lesion volume;

TWT 5 25-foot walk test.

Results are expressed as estimated mean monthly rate of change of each MRI variable (95% CI), p value related to the change within each group. a The ratio of change of the spinal cord area is calculated over a period of 2 years (from baseline to 24-month follow-up).

For the comparison between allele-positive and allele-negative patients: after adjusting for age and sex: b p , 0.1, c p , 0.01; after adjusting for age, sex,

and T2 lesion volume at baseline: d p , 0.05; after adjusting for age, sex, and EDSS at baseline: e p , 0.05.